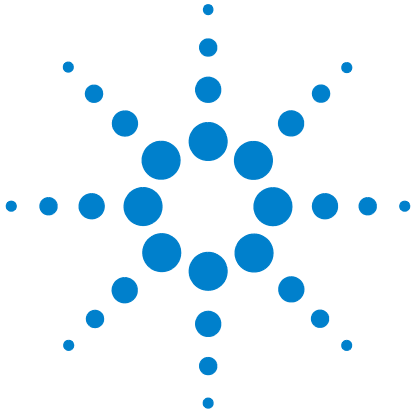

Agilent
GC Sampler 80/120
SPME Option

User Manual

Agilent Technologies

Notices

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1 Introduction

Prior to reading this manual the reader should first read the Agilent GC Sampler 80/120 User Manual (G6501-90000) and become familiar with the general operation of the PAL Autosampler including defining the position of objects and building methods and jobs.

The use of Solid Phase Micro Extraction (SPME) with the PAL Autosampler offers the analyst many features that will enhance the utility of this exciting sample preparation technique. These include:

1. Choice of 2,10, or 20 mL vials.
2. Large number of samples (Standard configuration is two trays with 98, 2 mL vials per tray or 32, 10/20 mL vials per tray. (Up to two additional trays can be added, if necessary.)
3. Agitation and heating of the sample during the extraction process.
4. Controlled heating time for each sample.
5. Each Agilent SPME fiber can be automatically pre-conditioned before a series of runs or after the desorption step in each run with an optional heating accessory.
6. Sampling and injection depth of the Agilent SPME fiber can be controlled automatically.
7. Conduct method development by using sequential methods withdifferentparameters(ie.incrementally increasing extraction times or extraction temperatures).

This manual covers the operation of the PAL Autosampler in the SPME mode with the basic software that is installed in the PAL Autosampler.

1 Introduction

2 Procedure for SPME Sampiling with the PAL AutoSampler

Preparation

Before beginning, the analyst should optimize the chromatographic conditions for the analytes to be analyzed — make sure an appropriate column, temperature program, and detector have been selected.

Injector liner

The injector liner is important in assuring good results when an SPME fiber is thermally desorbed.

Table 1 SPME/GC inlet liners

Description	Part number
Liner, inlet for SPME, deactivated	5188-6471

Injector Septum

The SPME fiber assembly includes a septum-piercing protective needle (Figure 1), which is either a blunt or tapered, hollow 23 gauge needle to protect the fiber coating during penetration of the septa.

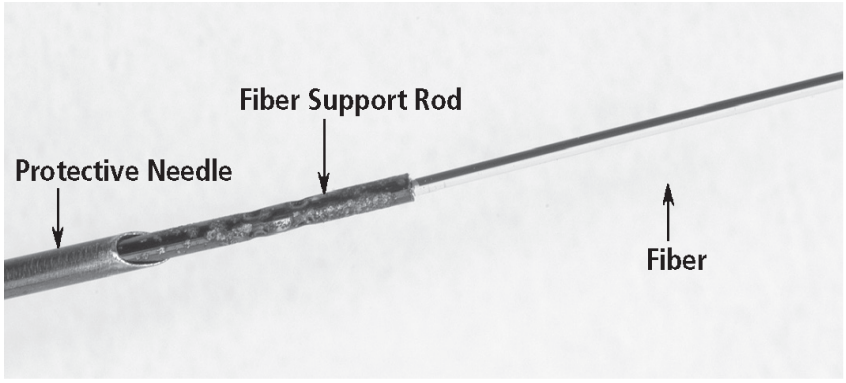

Figure 1 Detail of the fiber assembly

In comparison, liquid injection into a GC is usually accomplished with a tapered 26-gauge* needle. Therefore, sample introduction with a SPME fiber is more likely to result in septum failure. A septum-less injector seal, such as the Merlin MicrosealTM (Figure 2), is highly recommended.

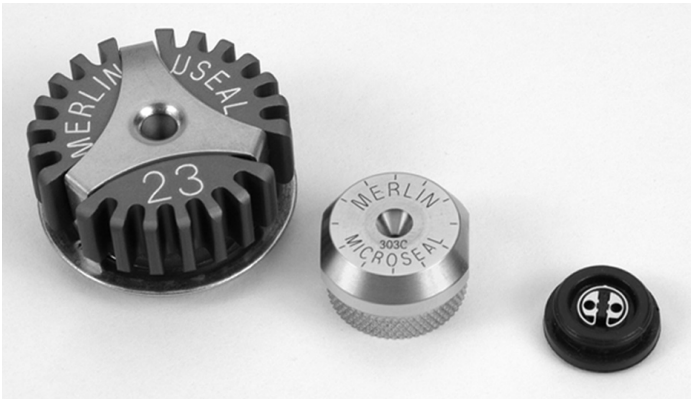

Figure 2 The Merlin Microseal can be istalled in a GC injector in place of a septum. The device contains a "duckbill" that allows a needle to enter the injector without leaking.

*The higher the gauge number, the narrower, the outer diameter.

Table 2 Merlin Microseal

Description	Part number
High pressure Merlin Microseal starter kit Includes microseal septum and nut	5182-3442
Microseal high pressure septum	5182-3444
Microseal high pressure nut	5182-3445

It is possible to use a conventional GC septum with SPME. To minimize septum failure, the following procedure is recommended:

1. Install a new septum. Pre-pierce the septum with a syringe needle first.
2. Puncture the septum with a SPME protective needle three or four times.
3. Remove and inspect the new septum. Pull off and discard any loose particles of septum material.
4. Reinstall the septum.
5. Metal fiber assemblies with 23 gauge needles will core septa more rapidly.
6. Check inlet liners for particles, replace liner if particles are present.

The user should monitor the head pressure on the column as the protective needle enters and leaves the injector to verify the integrity of the seal. A subtle leak will be indicated by shifts in retention time, no peaks or poor area count precision, or the presence of air in a mass spectrometer.

Injector temperature

Although temperature programmable injectors have become popular for minimizing decomposition of labile compounds and for eliminating discrimination based on volatility, SPME fibers are generally desorbed under hot, isothermal conditions. Rapid desorption from the fiber is necessary for sharp peaks without sample carryover. Injector temperature is normally 10-20 °C below the temperature limit of the fiber and/or the GC column. Typical inlet temperatures range from 220 °C to 310 °C.

NOTE Temperature programming causes peak broadening. The thermal desorption shall happen within milli seconds.

Sample vials

Many SPME applications will require the heating of the sample. For these applications, only vials recommended for the PAL Autosampler should be used. These vials are 10 mL and 20 mL with magnetic crimp-top caps and an 8 mm opening and capped with 1.3–1.5 mm thick septa. Do not use vials with a butyl rubber septa. Two mL vials with magnetic caps are also available, however, these are not recommended as the holes in the caps are small and fiber breakage is possible. For a complete list of vials, caps, and septa recommended for optimum SPME performance please refer to page 33.

Special adapters are required for the agitator when using 10 mL and 2 mL vials. The adapters for the 10 mL vials are shipped with the instrument. The 2 mL vial adapters are available as an option.

Selecting the appropriate fiber assembly

The appropriate SPME fiber for your analysis is based primarily upon three criteria.

1. Molecular weight, size and shape of the analyte(s)
2. Analyte polarity
3. Concentration range of analytes in sample

The data in Figure 3 provides general guidance on selection of fiber coating type based on the molecular weight range of the target analytes.

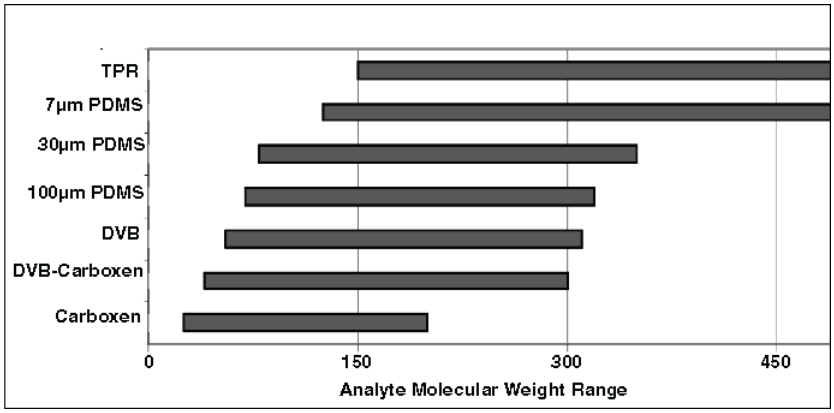

Figure 3 Fiber coating type based on molecular weight range

7 µm Polydimethylsiloxane (PDMS)	Absorbent	Nonpolar
30 µm PDMS	Absorbent	Nonpolar
100 µm PDMS	Absorbent	Nonpolar
85 µm Polyacrylate (PA)	Absorbent	Polar
65 µm PDMS-DVB, StableFlex™	Adsorbent	Bipolar
55 µm/30 µm DVB/Carboxen™-PDMS, SF	Adsorbent	Bipolar
85 µm Carboxen-PDMS, StableFlex	Adsorbent	Bipolar

Polar analytes are best extracted by polar fibers. Adsorbents are better for trace level of analytes with a more narrow linear range; whereas, absorbents are better for higher concentration levels and broader concentration level ranges.

Installing a fiber assembly into the SPME holder

When using the PAL Autosampler, we highly recommend the use of metal (superelastic assemblies). The second best choice are assemblies with 23 gauge needles. The wall of the 23 gauge needles is thicker and less likely to bend when piercing septa and when in the agitator.

1. Remove the barrel nut from the holder.
2. Push down on plunger so that plunger threads extend beyond the metal barrel.
3. Remove the assembly from the box.
4. Thread colored hub into the threads in the plunger and finger tighten until snug.

5 Push the plunger back so that brass flange on the assembly is touching the metal barrel.

6 Tilt holder so that the assembly needle is pointing upward (vertical).

7 Thread the assembly needle through the barrel nut by placing the nut over the vertical needle.

8 Gently shake the holder until the nut slides down to the metal barrel.

Setting up the PAL Autosampler for SPME

Refer to the Agilent GC Sampler 80/120 User Manual for installation of the PAL Autosampler and for setting the **x y z** parameters of the agitator, tray holders, trays, and GC injectors.

FiberExp position

In order for the SPME cycle to operate correctly, it is necessary for the injection unit of the PAL Autosampler to be positioned next to the agitator just before the extraction. This position has been designated as the **FiberExp** position.

From the **Job Queue** page, enter the following sequence:

Menu > **Setup** > **Objects** > **Vials** > **FiberExp**

Assuming the agitator is installed on the right side, set the **x**, **y** and **z** parameters so that the right edge of the injection unit is resting on the left rear edge of the agitator (Figure 4). If the agitator is on the left side, then the left edge of the injector unit should rest on the right rear edge of the agitator. Press **F4/Home**.

NOTE Do not position the round screw head from the needle guide directly on the edge of the agitator lid. With the side play of the lid, the needle guide could slip away.

Figure 4 Injection unit in the **FiberExp** position with the right edge just touching the left rear edge of the agitator

Installation of the SPME adapter

1. Press **F1/Menu** and then **F1/Chang Syr**. The injection unit will move to a position that will facilitate installation of the SPME adapter.
2. If the injection unit is directly over a sample tray, move the **Chang Syr** position.
3. Press **Continue** and then **Utilities** > **Syringe**.
4. Press **F3/Set Pos** and set the x y z positions to a location where there is a clear space under the fiber. Press **F4/Home** and repeat Step 1.

5 Install the plunger holder into the injection unit (Figure 5 left).

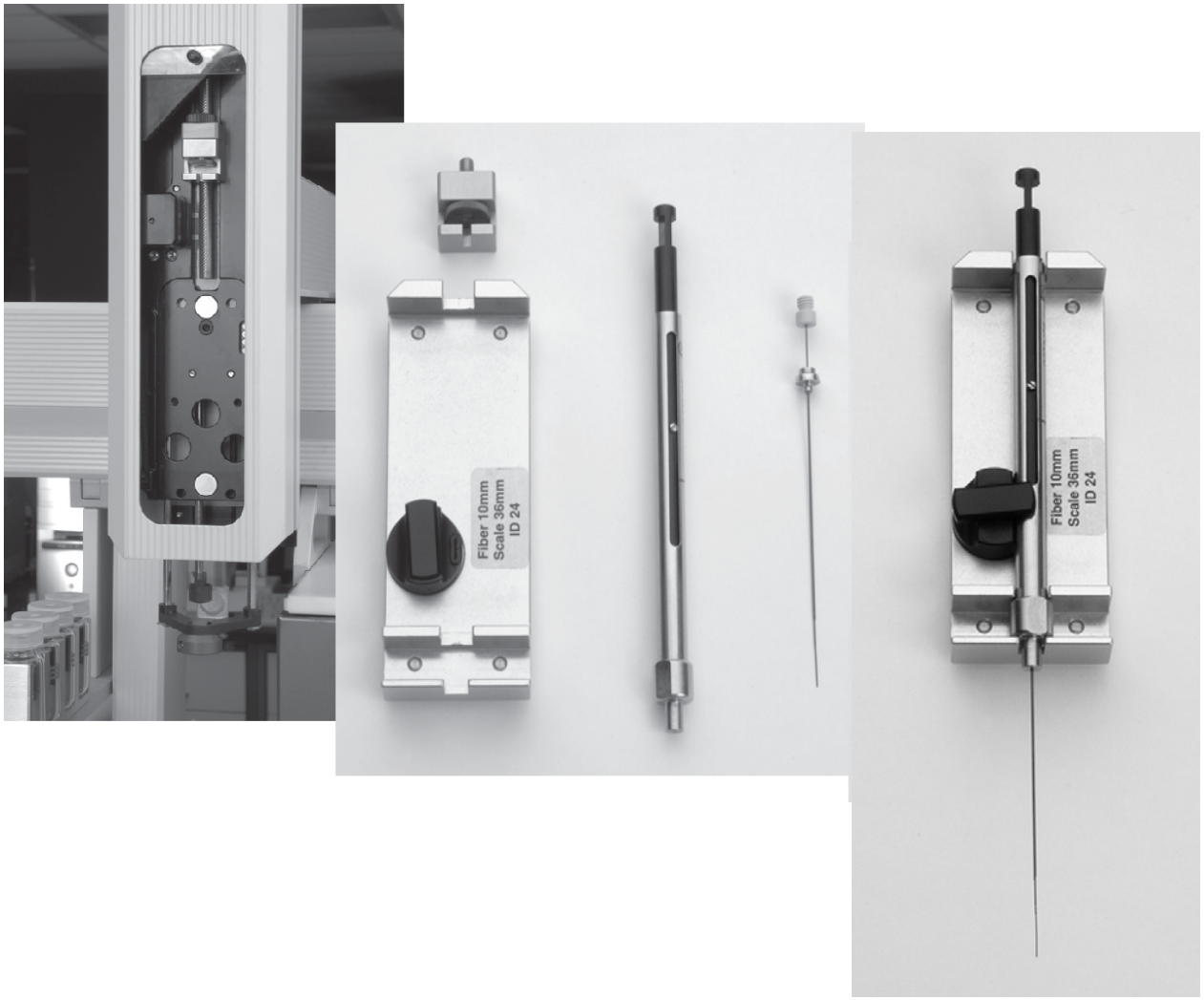

Figure 5 Left: injector unit with plunger holder installed, Center: unassembled kit components; Right: fiber holder and fiber assembly properly installed in the PAL Autosampler SPME adapter

6 Install a fiber assembly in the fiber holder and place it in the SPME adapter (Figure 5 right).

Pull up the plunger so that the fiber is completely withdrawn into the protective needle.

7 Place the SPME adapter, partially into the injection unit. In order to do this, tilt the top of the SPME adapter foreword slightly (Figure 6A) and thread the protective needle carefully through the upper and lower needle guides at the bottom of the injection unit. This process is made easier by

lifting the magnet so it is level with the needle guide, then push the needle through the opening (Figure 6B).

8 Push the plunger down so that approximately 1.5 to 2 cm of the fiber and fiber support rod are exposed.

9 Place the plunger crosspiece into the plunger holder. Allow the syringe adapter to “click” into place by magnetic force, against the syringe carrier.

10 Tighten the plunger retaining screw against the plunger crosspiece (Figure 6C) and press **Continue**.

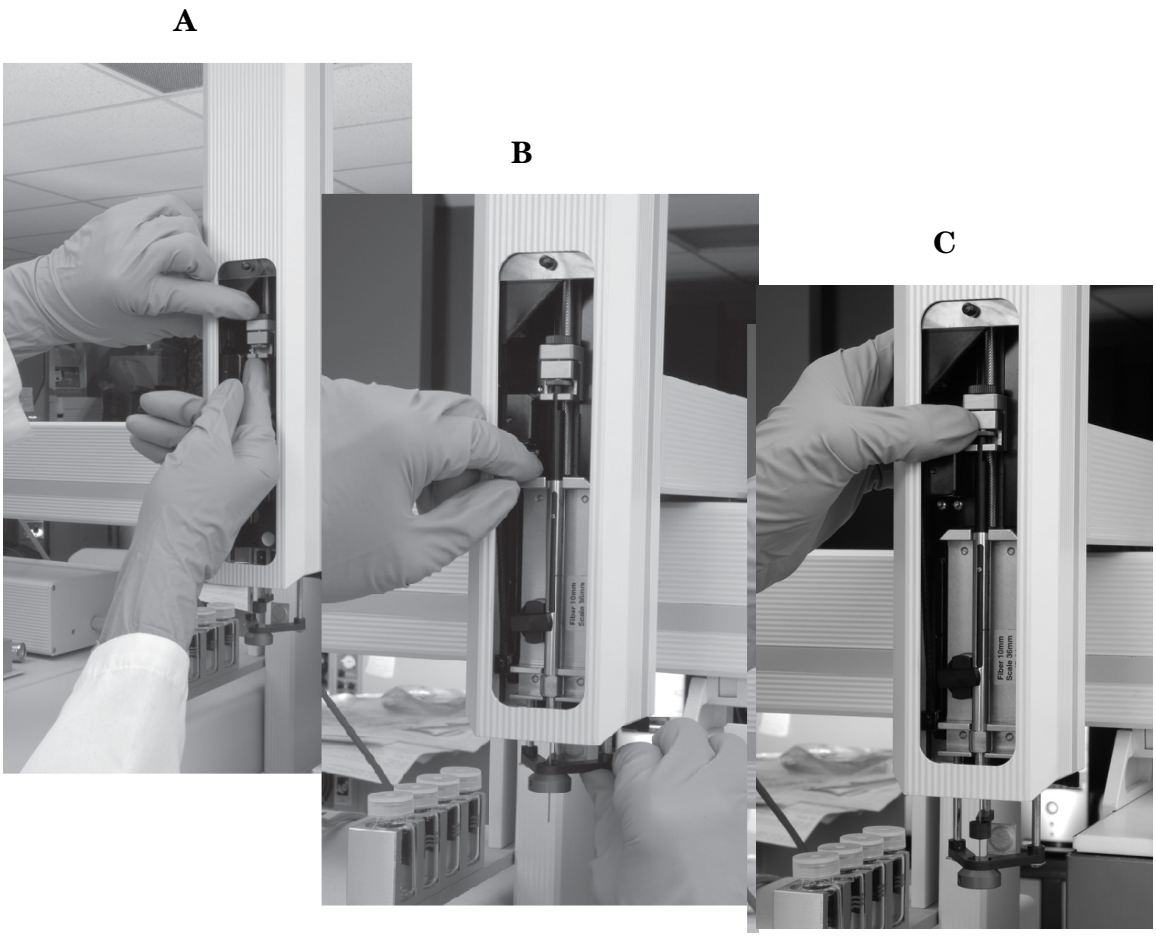

Figure 6 Installing the fiber holder assembly

NOTE Reverse the above procedure to remove the Agilent SPME fiber. Be sure to pull up the plunger of the fiber holder so that the unprotected fiber is not pulled through the upper and lower needle guides.

Standby position of the fiber

In this step, the end of the fiber is set so that it is just barely withdrawn into the protective needle. This will minimize coring when penetrating vial or injector septa. From the **Job Queue** page, enter the following sequence: **Menu** > **Utilities** > **Syringe.**

Scroll through the various parameters until you reach **Standby Pos** and press **Enter**. With your thumb, push up the lower needle guides until the end of the protective needle is visible. If the fiber is not exposed, turn the dial counterclockwise until you can see the fiber. Then turn the dial clockwise slowly, until the end of the fiber is flush with the end of the hollow tube. Turn the dial clockwise an additional 0.5 mm. Enter the value and press **F4/Home**.

Repeat this procedure whenever a fiber is installed.

Checking alignment of PAL Autosampler in the injectors

The PAL should have been initially aligned with the injection port during setup. This is accomplished without a needle in the injector.

The needle guide is used to position the unit. For SPME, the needle alignment is very critical.

1. Place the SPME adapter container holder and assembly into the PAL injector.
2. Follow the sequence in PAL menu setup injectors.
3. Select the appropriate injector and continue.
4. The needle will puncture the injection port (septum) nut opening.
5. Using **x**, **y**, **z** positions, align the PAL injector so the needle is straight in the **x** and **y** positions. This may require a very slight alignment, but it will assure that the needle will properly hit the center of the septum nut opening.

Setting fiber position in the injection port

The fiber position in the injection port is controlled by two parameters. The first parameter is the needle penetration depth. This is fixed for SPME at 33 mm. The second parameter that controls the fiber position is the **Injection Penetration**.

After you have set the injection position and made an injection with a fiber installed, verify that the fiber is intact after the injection. Press **F1/Menu** and then **F1/Change Syr** to view the fiber.

Injection penetration

Range (44–67 mm)

Injection Penetration is the distance that the fiber is pushed into the injection port with the plunger. The default setting is 54 mm, and this appears to be suitable for nearly all standard GC injection ports. The hot zones can vary between injection ports, so fine-tuning the distance can be obtained if desired. The key is to use the same depth for all of the analyses to insure consistency.

1 Remove the septum nut, loosen the injection port liner lock and remove the liner.

2 Place the liner against the septum contained inside the septum nut.

3 Mark the position on the liner that is the hot zone range. The manufacturer should report this distance from the top of the liner.

4 From the top of the hot zone range, measure 1 cm down into the liner. This is the point where you want the tip of the fiber to be placed. Note that this is the bottom of the fiber so 1 cm above the tip should be in the hot zone of the injection port.

5 Measure the distance in mm from the top of the nut to the point where you want the tip of the fiber to be in the injection port. This distance is the injection penetration distance.

6 Set this distance in the method and the fiber should be desorbing in the hot zone of the injection port.

NOTE For 2 cm. fibers, increase the injection penetration depth to the maximum value to fully expose the fiber.

Setting fiber depth in the sampling vial

The main parameter for controlling the depth into the vial is “vial penetration”. Vial penetration is the distance the plunger moves after the needle pierces the vial. The distance that the needle pierces a vial is fixed at 11 mm.

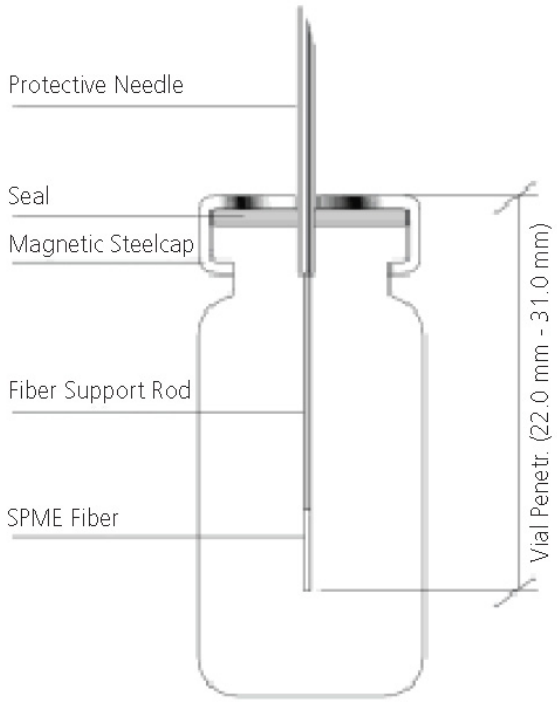

Figure 7 Showing the “Vial penetr” parameter

Vial penetration

Range (22–31 mm)

Vial penetration determines how far the fiber extends into the vial. At 22 mm, a 1 cm fiber is fully exposed; however, the plunger can be further extended to adjust the depth of the fiber in the vial. When using a 2 cm fiber, the vial penetration must be set at 31 mm to fully extend the fiber. Vial penetration is usually set in the method. If you do not have the software, create an “SPME Test” method in the PAL controller. Since it is not possible to see the fiber depth in the agitator, set the fiber depth out of the tray. Use the same depth when the vial is in the agitator.

Prior to setting the injection penetration, the sample volume

must be determined, especially for direct immersion into a liquid. See Table 1 for recommended volumes.

Table 3 Recommended volume sample for various vial volumes and fiber length

Vial volume	Headspace sampling 1 cm fiber length	Headspace 2 cm fiber	Direct immersion in liquid 1 cm	Direct immersion in liquid 2 cm
2 mL	0.4–0.6 mL	NR	1.2–1.3 mL	NR
10 mL	4.0–6.0 mL	NR	9.0 mL	NR
20 mL	10–15 mL	10–13 mL	18 mL	18–19 mL

1. Place the vial, filled to desired liquid volume, in the 8th position of the 32 vial tray (14 position of 98 vial tray). Be sure that for 32 vial tray that the PAL has been set for the proper Traytype (vial size).
2. Using the Agilent FSE Driver **Run SPME out of Tray** method, select the proper tray and vial position. You may want to increase your extraction time to 1 min. or more.
3. Check the fiber depth in the sample. For direct immersion in liquid, you want the entire fiber, except the top 1 mm, immersed in the liquid as shown in Figure 7.
4. Adjust the “vial penetration” setting in the software until the fiber is in the proper position. This may take several attempts. The depth will remain the same in the agitator.
5. For headspace, ensure that the fiber is not touching the liquid and is centered in the headspace.
6. Follow step 4 until the desired depth is obtained.

Using the SPME agitator/heater

The SPME Agitator can be used to heat samples, and to agitate samples. Either function can be used independently of the other. The agitator can be used for 2 mL, 10 mL and 20 mL vials. Inserts are available to fit in the six agitator positions to handle 2 mL and 10 mL vials. The agitator speed when using SPME is fixed at 250 rpm. Faster speeds can be used during pre-incubation, but are not recommended during the fiber extraction process. After the incubation temperature has been determined, you can set the **Standby temperature** of the agitator. This will keep the temperature constant during sampling.

Menu > Utilities > Tray > Agitator > Standby Temp

Parameters of agitator

Incubation time

0 to 1440 minutes

Agitator speed

250–760 rpm (Speed for pre-incubation, speed set at 250 with fiber inserted.)

Agitator on time (0–99 s)

The agitator rotates in intervals. The time that the agitator rotates in one direction is “time on”. During extraction with SPME fiber, the agitation is only in one direction and is on the entire time.

Agitator off time (0–99 s)

This is the amount of time that the agitation is off between intervals.

For direct immersion of liquid samples

If direct immersion is required to extract analytes of interest, an agitator can be used to shorten the extraction process. Usually heat is not used when direct immersion is being used. However, you may want to set the agitator temperature to 30 °C, to keep consistency between samples. For analytes with low volatility, some analysts will heat the sample to shorten the extraction time.

For headspace sampling of liquids or solids

If samples have sufficient volatility, headspace sampling is usually a better choice than immersion. Samples extract more rapidly in headspace, the fiber remains clean, and usually less interfering peaks are observed.

For most samples, heat will be applied. For liquid samples, agitation may be used along with heating. For solid samples, agitation may not be necessary with heating of the sample. Optimize heating and time by designing multiple methods. First select a fixed time and vary temperature. Typical temperature range is 35 °C to 70 °C. Rarely is the extraction temperature set above 70 °C, because the increased temperature will desorb

analytes off the fiber and may drive more water into the fiber and needle sheath. After the optimum temperature is obtained, select an appropriate extraction time. Keep in mind the total GC cycle time when setting extraction times.

Optional bakeout of the fiber after injection

With some fibers, a high temperature is necessary to desorb the analytes completely. Often, the GC injector cannot be set to a high enough temperature because a column with a low temperature limit is installed. With the PAL Autosampler, the user can bake the fiber after desorption in a separate bakeout station (Figure 8). This is an optional piece of hardware.

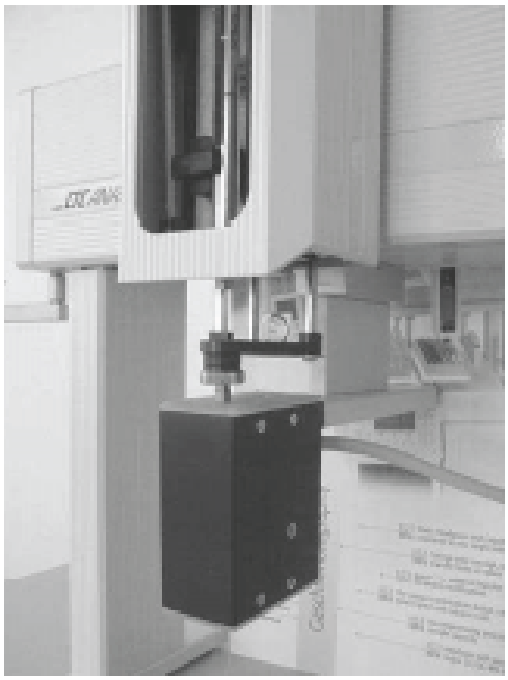

Figure 8 Optional bakeout station. The baking occurs with a flow of inert purge gas.

To enable this feature, install the bakeout station. Then define the position of the bakeout station (**NdlHeater**) as follows:

Menu > **Utilities** > **Injector** > **NdlHeatr**

Set the x y z parameters. The path is mixed up. Temperature/XYZ positions are reversed.

The temperature can be set in increments of 5 °C, from 30 °C to 350 °C. To set the temperature, Press:

Menu > **Setup** > **Objects** > **Injector** > **NdlHeatr**

Building an SPME method

See the Agilent GC Sampler 80/120 User Manual for details on how to build a method.

The parameters in the SPME method are discussed in Table 4:

Table 4 Parameters in the SPME method

Parameter	Value	Comments
Cycle		SPME
Syringe		Fiber
Pre Inc time	00:00:00–23:59:59	Allows the sample to be preheated prior to insertion of the fiber.
Incubat temp	30.0 °C –200 °C or **OFF**	**OFF** for "Sampling out of the Tray" at ambient temperature without agitation. A maximum of 80 °C is suggested.
Agi On time	0s–99s	Set "0s" to turn off agitation. Used for "Sampling out of Tray"
Agi Off time	0s–99s	
Vial Penetr	22.0–31.0 mm	Distance from top of vial septum to end of fiber (Figure 7)
Extract time	00:00:10–23:59:59	Sampling time in liquid or headspace
Desorb to	None–Waste 2	Normally an injector such as GC Inj1, GC Inj2 or "Front" or "Rear" is entered here.
Inj Penetr	44.0 mm–67.0 mm	Distance from top of injector nut to end of fiber
Desorb time	00:00:10–23:59:59	Time in injector
Fiber bakeout	00:00:00–23:59:59	For baking out the fiber after desorption in the optional bakeout station. If "0" , the unit does not move to the bakeout station.
GC runtime	00:00:30–23:59:59	Enter the complete GC cycle time including cool-down and re-equilibration to coordinate the PAL Autosampler and GC cycles.

After the incubation temperature is determined, it is convenient to set the standby temperature of the agitator to this temperature.

Enter the following sequence from the **Job Queue** page:

Menu > Utilities > Tray > Agitator

Scroll down to **Standby Temp** and set the temperature.

Running the SPME test sample

The SPME Sensitivity Test Sample is composed of 1 mL each of nitrobenzene and nitrotoluene in water (1% methanol has been added to stabilize the sample). These compounds were selected because they exhibit a good response with many GC detectors, including the flame ionization detector, electron capture detector, thermionic selective detector, and the ion trap detector. A representative SPME test sample chromatogram is shown in Figure 9.

Sample	SPME Evaluation test mix
SPME fiber	100 µm PDMS Fiber
Extraction	10 min. immersion in 4 mL vial, ambient, no agitation
Desorption	2 min. at 240 °C
Column	15 m x 0.25 mm x 1.0 µm
Oven	60 °C (1min.) to 200 °C at 20 °C/min.
Detector	FID
Carrier gas	helium, 40 cm/sec. at 60 °C, constant pressure
Injection	240 °C, splitless/split, closed inital 0.5 min. then vented at 50 mL/min.

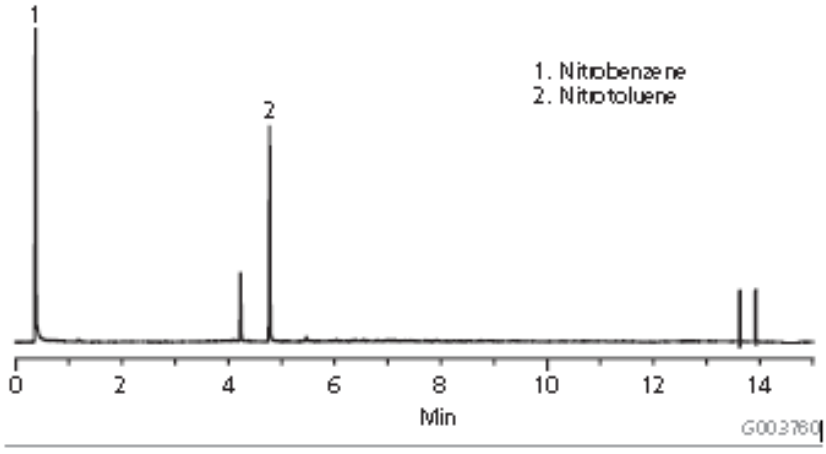

Figure 9 Chromatogram of the SPME test sample

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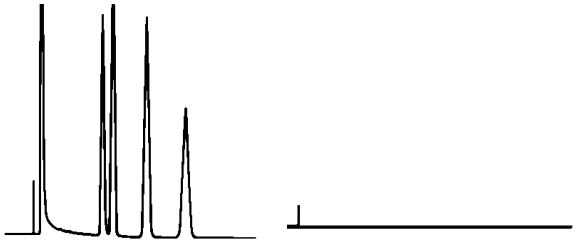
This is a quick reference to symptoms and possible causes of the most common problems experienced by users.



Normal **Problem**

No peaks seen in GC analysis

Possible cause	Remedy
Instrument problems*	Inject standard mixture detector response, see GC troubleshooting guide for further assistance.
The splitter vent was left open.†	Run splitless injection for 2 minutes.
The analyte concentration is too low to be detected.‡	Start with known concentration (1 ppm) of analyte in de-ionized water mixture. Optimize extraction by adjusting extraction time, temperature, and chemical condition of pH and salt.
Solvents present in sample competing with SPME extraction.‡	Minimize solvents in the sample to <3% by dilution in water.
Headspace volume too large to establish equilibrium with fiber.‡	Reduce headspace to 50% or less, agitate sample vigorously, or increase the sampling temperature.
Coating on fiber deteriorated.**	Replace fiber. Fibers are reusable and will last for 50 injections on average.
Incorrect SPME fiber used for extraction.**	This is beyond the scope of this guide. Please contact technical service (800-359-3041) for assistance if you are experiencing problems selecting the appropriate fiber for your application.
There is a leaking injection port (septum or connection).†	Replace septum and tighten nut properly.
There is a leaking sample vial.‡	Replace vial septum and seal cap properly.
Loss during transport from the field.‡	Move the depth adjusting lever of the portable field sampler to the top-most locking slot, so the end of the septum-piercing needle is totally withdrawn into the sealing septum of the sampler. If the fiber will be stored for more than one day we recommend that it be stored at subambient temperature. This reduces the chance of breakdown and loss of sample that could occur at higher temperatures.

* Analysis related

† Desorption related

‡ Sampling related

**Product related

Extraneous peaks in analysis

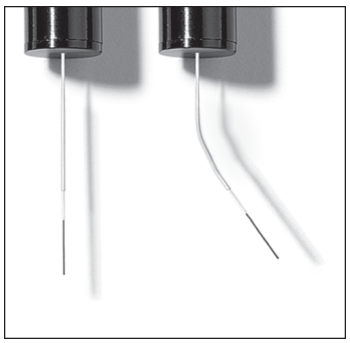
Possible cause	Remedy
Septa used in sampling vial or injection port is outgassing organic contaminants.* †	Prebake the vial septa for 2 hours at 68 °C prior to use. Use low-bleed LB-2 septa to minimize injection port septum bleed.
Fiber is not preconditioned prior to sampling.‡	Precondition fiber at the recommended conditioning temperature in the fiber instruction sheet. Once fiber is preconditioned, only 1–2 minutes is required to clean the fiber prior to sampling.
Inlet liner is contaminated or contains septa particles.**	Replace the inlet liner. Use pre-drilled septum or a septumless injector system (e.g. Merlin Microseal).
GC column is collecting analytes on the front of the column because it is not heated high enough in sample analysis.*	Complete GC analysis temperature program before injecting another SPME extract and keep column at 150 °C when not in use.
Interfering peaks coelute with analytes of interest.*	Change GC column or temperature program.
Carryover from previous analysis of the fiber.‡	Bake out fiber at the recommended conditions for several additional minutes.
Cross-contamination from laboratory air.*	Do not expose the fiber to the laboratory environment at any time during the sampling or injection steps. Analyze control blanks using the same handling process as the sample to determine if technique or laboratory cross-contamination is present.
Cross-contamination during transport from the field.*	Move the depth adjusting lever of the portable field sampler to the top-most locking slot, so the end of the septum-piercing needle is totally withdrawn into the sealing septum of the sampler. If the fiber will be stored for more than one day, we recommend that it be stored at subambient temperature. This reduces the chance of sample cross-contamination.

* Sampling related

† Analysis related

‡ Product related

**Desorption related



Needle bends during injection into sample vial or GC injection port

Possible cause	Remedy
Improper manual sampling technique.*	To prevent the needle from bending when doing manual SPME sampling: **1** Adjust the SPME needle to the 0.2 depth gauge setting on the plunger (first tickmark). This will expose about 3 mm of the needle through the end of the black holder. **2** Hold the SPME assembly on top of the sampling vial with the bottom of the black holder flush with the top of the vial cap. **3** Hold the sampling vial and black SPME holder base securely with one hand. Twist the stainless steel plunger clockwise with the other hand. **4** Keep turning the plunger until the desired depth setting is achieved (you will usually hear a pop when the needle pierces the septum). **5** Expose the fiber and perform sample desorption as usual.
Improper manual desorption (injection) technique.†	To prevent the needle from bending when doing manual SPME injections: **1** Adjust the SPME needle to the 0.2 depth gauge setting on the plunger (first tick mark). This will expose about 3 mm of the needle through the end of the black holder. **2** Position the SPME assembly on top of the GC injection port or in the SPME inlet guide with the bottom of the black holder flush with the top of the injector or guide. **3** Hold the black SPME holder base securely with one hand. Twist the stainless steel plunger clockwise with the other hand. **4** Keep turning the plunger until the desired depth setting is achieved (you will usually hear a pop when the needle pierces the septum). **5** Expose the fiber and perform sample desorption as usual.
Vial or injection port septum is too tight.* †	Slightly loosen the vial closure or injection port nut
Septa in sample vial/injection port is too thick or coated with thick Teflon coating.* †	Use LB-2 septa for injection port or silicone septa with <10 mil Teflon on sampling vials. Shorten the amount of exposed needle on SPME holder to 0.5 inch or (~1 cm) before puncturing the vial septa. Adjust the holder needle setting to the desired depth for sampling. Do not use butyl rubber style septa.

* Sampling related

† Desorption related

Needle bends with automated injection system

Possible cause	Remedy
GC inlet liner is too narrow or packed with adsorbent material.*	Use larger splitless inlet liners (0.75 mm id or larger) without glass wool or adsorbents.
Needle is out of alignment with injection port or sample vial.† *	Reference autoinjector manual on alignment.

* Desorption related

† Sampling related

Fiber will not retract or sticks in holder needle

Possible cause	Remedy
The end of the needle is plugged with a piece of septum.*	Injection port septum nut is overtightened. Loosen the nut slightly to allow for improved injection. Use pre-drilled injection port septa or a septumless injector system (for example Merlin Microseal).
The fiber was exposed to solvents that caused swelling of coating.†	Do not expose PDMS coated fibers to nonpolar solvents such as pentane, methylene chloride, or diethyl ether. Do not expose Carbowax fibers to polar solvents.
The top screw in the holder assembly is too tight.‡	Loosen the top screw on the holder assembly slightly to allow for free movement of the plunger.

* Desorption related

† Sampling related

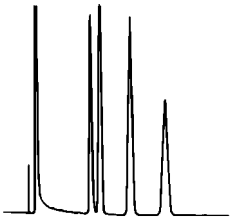
‡ Product related

Fiber breaks

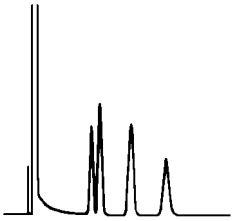
Possible cause	Remedy
Fiber was not retracted into the protective needle after removal from sample vial or injection port.* †	Retract fiber into protective needle during insertion into vial/injection port and removal.
The end of the needle is plugged with a piece of septum.†	Injection port septum nut is overtightened. Loosen the nut slightly to allow for improved injection. Use pre-drilled injetion port septa or a septumless injector system (for exampleMerlin Microseal).

* Sampling related

† Desorption related



Normal



Problem

Reproducibility is poor

Possible cause	Remedy
Time and temperature variations during sampling.*	Time of extraction and temperature are the two most critical conditions to control. Use a timing device and a calibrated thermometer to ensure reproducible results. Remember that room temperature fluctuations will influence the ambient sample temperature.
Not consistently positioning the fiber at the same depth during sampling.*	Position fiber just below sample surface for immersion sampling and at a consistent position above the sample during headspace sampling.
pH or salt conditions varying during sampling.*	Apply any pH or salt adjustments made to the samples uniformly across all extractions .
Equilibrium is not reached during extraction.*	Determine minimum time for equilibrium using a standard mixture and controlled extraction conditions. Note that full equilibrium is not required to be reached for all applications to achieve reproducible results.
Varying organic content in the samples.*	Dilute samples to minimize solvent interference or use headspace sampling to minimize solvent effect. Use internal standards, surrogates, or the standard addition technique to compensate for variations in sample matrix.
Varying headspace in sample vials during headspace extraction	Minimize headspace volume to 50% or less and agitate the sample. Maintain the same headspace volume and agitation conditions across all extractions.
Solid samples not releasing analytes for extraction	Grind solid into small particles, add to water, and apply heat and agitation.
Competing analyte displaces compound of interest/or interferes.*	Reduce the extraction time to minimize displacement/or interference.
Not reproducing desorption conditions.†	Verify that the fiber position (depth), desorption time, temperature, and splitless conditions are consistent. Use an automated SPME system to improve reproducibility.

Reproducibility is poor

Not using agitation during sampling or apply it inconsistently.*	Use a stir bar or soncication system to agitate the sample during sampling. Maintain consistent agitation conditions for all standards and samples.
Sample volumes are inconsistent.*	Maintain consistent volumes for all standards and samples.

* Sampling related

† Desorption related

Fiber discolored

Possible cause	Remedy
Fiber is oxidized during fiber conditioning orsample injection into GC .* †	Minimize oxygen in carrier gas, condition fibers in oxygen free gas flow. Reduce the injection port temperature to the recommended maximum setting. Carbowax/DVB coatings are especially sensitive to temperature (<260 °C is recommended).
Heating during injection.† ‡	Does not usually affect the performance of the fiber. Always minimize the oxygen content in the GC carrier gas to avoid oxidizing the fiber coating. The polyacrylate coated fiber will discolor above 280 °C. Carbowax/DVB may slightly darken during use, however, if the fiber turns brown, lower the injection port temperature (265 °C is the recommended maximum) and check the system for leaks.

* Sampling related

† Desportion related

‡ Product related

Number of injections from the fiber is less than previously obtained

Possible cause	Remedy
Fiber is oxidized during fiber conditioning or sample injection into GC.* †	Minimize oxygen in carrier gas, condition fibers in oxygen free gas flow. Reduce injection port temperature to the recommended fiber maximum.
Coating on fiber deteriorated.‡	Replace fiber. Fibers are reusable and will last for 50 injections on average.
Fiber was exposed to solvents that cause swelling of coating.*	Do not expose PDMS coated fibers to nonpolar solvents such as pentane, methylene chloride, or diethyl ether. Do not expose Carbowax fibers to polar solvents.

* Sampling related

† Desorption related

‡ Product related

Fiber breaks in injector

Possible cause	Remedy
Improper depth in injector.	Verify (see above) that the bottom of the SPME fiber syringe is not less than 5 mm into the insert.
Septum corings or other particles are in the injector.	Replace insert. If septum particles are present, consider using a seal such as the Merlin Microseal to eliminate the septum.

Poor precision

Possible cause	Remedy
Vials are leaking.	Verify that the cap cannot be turned after sealing. Reduce the extraction temperature to see if the precision improves. Temperatures > 80 °C are not recommended.
Poor sample handling.	See the Advantage Note on SPME method development in the "SPME Application and Advantage Note" section of this manual.

Sample carryover

Possible cause	Remedy
Fiber is not fully desorbed.	Increase desorption time and/or temperature or bake out the fiber after each injection.
Fiber support rod is submerged in liquid sample.	Reduce the fiber penetration depth in vial or reduce the amount of sample in the vial.

Extraneous peaks in blanks

Possible cause	Remedy
Contamination is in the GC.	Verify that the GC is clean by making a run without injecting a sample.
Contamination is in the sample vial septa.	Sample an empty vial without a septum installed. Sample an empty vial with a septum installed. If the contamination is from the septum, bake the septum in a laboratory oven at 150 °C overnight. This will minimize extraneous peaks. Vial is not completely released from the needle when the injection unit moves away. Replace short tension cord (PM).
Fiber bends in Agitator.	Belt from Agitator is worn out or even broken. Replace belt (O-Ring) from Agitator, PM.

4 Supplies

Sampling vials for PAL Autosampler Headspace

Based on studies involving Agilent SPME fiber durability with the PAL Autosampler system, it is important that thinner septa be used to seal vials for SPME use. We recommend a 1.5 mm silicone PTFE lined septa with SPME neck vials or screw cap vials. A steel cap should be used with screw top vials and either aluminum/tin or steel can be used with the SPME neck vial. A 1 mm thick Viton septa works best with screw cap vials. For SPME use, we strongly recommend that caps with a 8 mm hole be used instead of the 5 mm hole.

Table 5 Sampling vials

Description	Quantity	Catalog number
Crimp vial, 2 mL clear wide opening	100	5181-3375
Crimp vial, 11 mm magnetic, silicone/PTFE septa	100	5188-5386
Crimp vial, amber glass 0.7 mL	1000	5183-4487
Crimp cap, 8 mm silicone/PTFE	500	5180-0842
Screw top vials, 2mL, clear wide opening	100	5182-0714
Blue screw caps, PTFE/silicone septa	100	5182-0720
Snap top vial, 2 mL, clear wide opening	100	5182-0544
Blue snap cap, PFTE/silicone septa	100	5182-0541

4 Supplies

5 References

Useful web pages

- www.agilent.com/chem
- University of Waterloo (SPME research) http://spme.uwaterloo.ca/,
- University of Texas (SPME bibliography) http://www.cm.utexas/edu/~brodbelt/spme_refs.html

Books

1 J. Pawliszyn, **Solid Phase Microextraction: Theory and Practice**, Wiley-VCH, Inc, New York, 1997

2 S. Wercinski, editor, **Solid Phase Microextraction: a Practical Guide**, Marcel Dekker, New York, 1999

Trademarks

CarbowaxUnion Carbide Chemicals & Plastics Technologies Corp.
Carboxen, StableFlex, Thermogreen—Sigma-Aldrich Co.
Microseal—Merlin Instrument Company
Viton—E.I. duPont Nemours & Co., Inc.

5 References

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